The examiner's suggestion for rewording the sections of claims 1 and 27 that precede the Markush group listings has been made.

The rejection of claim 1 as indefinite for including parts of an antibody molecule other than an Fc portion in the list of isotype combinations is respectfully traversed. By definition, the isotype of an antibody is characterized not only by the Fc-region (the CH1 and CH2 domains) but also by the hinge region and the CH1 domain. The fact that the sequences of an antibody isotype mostly start from the CH1 region is reported in the literature in the field, examples of which are Schreier, P.H., et al., "Multiple differences between the nucleic acid sequences of the $IgG2a^a$ and $IgG2a^b$ alleles of the mouse," *Proc. Natl. Acad. Sci. USA* **78**(7):4495-4499 (1981); Brüggemann, M., et al., "Sequence of rat immunoglobulin γ_{2c} heavy chain constant region cDNA: extensive homology to mouse γ_3," *Eur. J. Immunol.* **18**:317-319 (1988), copies of which are enclosed herewith in the accompanying Information Disclosure Statement. In view of this understanding in the state of the art, therefore, each of the members in the Markush group list indeed defines an isotype combination.

The rejection of claim 1 as indefinite for its use of the term "heterologous" is respectfully traversed. It is indeed possible to create bispecific antibodies that are non-heterologous (i.e., homologous). The term "heterologous" refers to a characteristic of the constant portions of the antibody molecule, and homologous bispecific antibodies can be created from identical subclasses of half antibody molecules (i.e., those with identical constant portions) that have different binding sites.

The rejection of claim 1 for its recitation of a vaccine product is obviated by the amendment above which replaces "vaccine" with "antibody-tumor cell preparation."

The awkwardness of the wording in claim 1 that arose from the expression "capable of activating the Fc receptor-positive ..." is obviated by the above amendment which sets out the four properties of the bispecific antibodies in separate paragraphs.

The examiner's suggestion regarding claim 13 has been adopted.

Claim 14 has been reworded to clarify the recitation that the tumor cells, the intact heterologous bispecific antibodies, and the peripheral blood mononucleated cells are all incubated together in a single step. This is properly dependent on claim 1 by narrowing the

scope of the corresponding step of claim 1 to limit the incubation to require the combined presence of the three components while claim 1 only requires that two of the three be present.

The perceived lack of antecedent basis for claims 23, 25 and 26 is obviated by the amendment to claim 1 to recite "an antibody-tumor cell preparation." Claim 25 is canceled by this amendment.

The rejection of claim 27 for lack of antecedent basis is respectfully traversed. as recognized by Examiner Helms in the telephonic interview of December 18, 2001, antecedent basis does in fact exist in claim 1, since the last member of the Markush group list is indeed "rat/mouse."

Claim Rejections – 35 USC 112, First Paragraph

The rewording of claim 23 by the above amendment clarifies the invention intended to be recited in this claim, which is the prevention of further tumor disease in a subject in which cancer has already occurred, using antibodies directed against the tumor cells that arose in the patient. The claim is not intended to cover methods of prevention of tumor disease in a subject in which cancer has not already occurred. As the examiner may have noted, the use and effectiveness of this method are demonstrated in Example 2 of the specification, in which animals were initially injected with tumor cells, then treated with an antibody-tumor cell preparation recognizing a target on the tumor cells of the initial injection. After 144 days, a second injection of tumor cells was made. By then, tumor cell immunity had developed in all of the animals treated with the initial injection of tumor cells and accordingly all of these animals survived the second tumor cell injection. Thus, with the clarified claim wording and the experimental procedure and results reported in Example 2, Applicants submit that claim 23 recites subject matter that is indeed described in the specification in accordance with the requirements of 35 U.S.C. 112, first paragraph, and reconsideration of this rejection is respectfully requested.

Claim Rejections – 35 USC 103(a)

The rejection of claim 27 as obvious over the combination of Volker et al., Deo et al., and Lindhofer et al., is respectfully traversed.

Applicants' invention utilizes one antibody molecule that binds three different cell types (tumor cells, T-cells, and Fc receptor positive cells) simultaneously to achieve an antibody-tumor cell preparation for anti-tumor vaccination. Volker et al. teach the use of antibodies that bind to viral antigens on tumor cells. To obtain these antibodies, the tumor cells are modified, notably by the introduction of a virus protein of Newcastle Disease Virus (NDV).

The introduction of further antigens, particularly viral antigens, into a tumor cell is highly undesirable for reasons of safety. As a result, clinics generally prefer to use applications that are virus-free. The teachings of Volker et al., particularly that the use of Newcastle Disease Virus is absolutely necessary for a successful vaccination, are thus contrary to the conventional wisdom in clinical practice.

The present invention uses a tumor antigen on a tumor cell as a target for the bispecific antibodies. The creation of a transgenic tumor cell by introducing a viral antigen into a tumor cell is thus neither necessary to Applicants' invention nor indicated in any part of Applicants' specification or claims. Thus, viral contamination and its potential side-effects are avoided.

Since the Volker et al. disclosure is silent on the use of bispecific antibodies that have anti-Fc-receptor specificity, the Office Action cites the Deo et al. disclosure as a teaching of the usefulness of Fc-receptor specific antibodies. Applicants submit however that this combination of the two patents fails to lead one skilled in the art to the present invention.

The focus in the teaching of Volker et al. is the use of an antibody with two binding components, one directed against the cell surface protein which is a virus protein, and the other against a costimulatory acting molecule on an effector cell which is a T-cell. The teaching emphasizes the use of portions of antibodies, namely Fab', $(Fab')_2$, F_v or $(F_v)_2$ fragments. This is contrary to the teachings of the present invention in which a critical feature is the use of intact bispecific antibodies. Applicants' invention is further limited to intact bispecific antibodies that simultaneously have four distinct characteristics, one of which is the ability to activate Fc-receptor positive cells to induce or increase the expression of cytokines or costimulatory antigens. Only certain subclasses of intact heterologous antibodies have these

characteristics. Neither Volker et al. nor Deo et al. offer any suggestion of how to select these subclasses, nor does either reference suggest the desirability of preparing an antibody-tumor cell preparation, much less a motivation to use antibodies that have these characteristics to prepare such a preparation.

The disclosure of Deo et al. also emphasizes the use of antibody fragments. The combination of Volker et al. and Deo et al. thus leads one skilled in the art to the use of antibody fragments rather than intact bispecific antibodies. Even if one skilled in the art were to attempt to select a bispecific molecule with specificities for a tumor antigen, a costimulatory molecule on an effector cell, and an anti-Fc-receptor, from among the various alternatives presented in Volker et al. and Deo et al. disclosures, and do so contrary to the preferred teachings and embodiments of these references, neither reference provides any guidance for making such a selection, or any bispecific molecule that would have these specificities and also be capable of activating the Fc-receptor positive cell to induce or increase the expression of cytokines or costimulatory antigens.

The nonobviousness of the isotype combinations of this invention is further demonstrated by ability of these combinations to demonstrate the features listed above without adding costimulatory antigens or cytokines from external sources, as taught for example by Honsik et al., U.S. Patent No. 4,844,893 (cited by the examiner on Form PTO-892 of Paper No. 9). Bispecific molecules of the present invention are able to bind and simultaneously activate accessory cells such as macrophages via their Fc-region leading to an appropriate T-cell costimulation and activation. As reported in Example 1 of Applicants' specification in the paragraph bridging pages 28 and 29, the accessory cells activated by the trifunctional bispecific antibodies of the present invention via the Fc-region are able to deliver important costimulatory signals to the T-cells.

Studies performed by the inventors herein and their coworkers, published subsequent to the filing of the present application, have confirmed that the subclass combinations of this invention are indeed able to effect an efficient tumor cell treatment while also inducing an anti-tumor immunity. These reports appear in Zeidler, R., et al., "Simultaneous activation of T cells and accessory cells by a new class of intact bispecific

antibody results in efficient tumor cell killing," *J. Immunol.* **163**:1246-1252 (1999); Zeidler, R., et al., "The Fc-region of a new class of intact bispecific antibody mediates activation of accessory cells and NK cells and induces direct phagocytosis of tumor cells," *British J. Cancer*, **83**(2):261-266 (2000); and Ruf, P., et al., "Induction of a long-lasting antitumor immunity by a trifunctional bispecific antibody," *Blood* **98**(9), 2526-2534 (2001). The veracity of the reports in these documents, which are not prior art relative to the present invention, is established by the enclosed Declaration of Dr. Horst Lindhofer Under 35 U.S.C. 132.

The three documents cited in the preceding paragraph also demonstrate that the activation of T-cells, for example, via the CD3 binding arm of the bispecific antibody, and the costimulation via the activated accessory cell in the vicinity of the tumor cell lead to a very efficient tumor cell killing, an unexpected result which is not obvious from the teachings of Deo et al. and Volker et al.

The deficiencies of the teachings of Volker et al. and Deo et al. are not overcome by the disclosure in the Lindhofer et al. reference (*J. Immunology*, 1995) that is cited by the Office Action as prior art. This 1995 Lindhofer et al. reference merely discloses a method for a single-step purification of bispecific antibodies and offers no guidance toward use of any of the bispecific antibodies for an antibody-tumor cell preparation.

None of the references offer any guidance for selecting intact bispecific antibodies or for using intact bispecific antibodies in the preparation of either an antibody-tumor cell preparation or a vaccine as recited in Applicants' claims. Either individually or in combination, therefore, the references fall short of providing one skilled in the art with any reasonable expectation of success for the induction of an anti-tumor immunity. The purification of bispecific antibodies is quite different from their use in the methods presently claimed.

Claim Rejections – 35 U.S.C. 112, First Paragraph

The rejection of claims 1-8, 13-21, 24-26, and 28 for lack of enablement in the specification commensurate with the scope of the invention recited in these claims is respectfully traversed.

The Office Action states that Applicants' working example does not indicate whether the exemplified bispecific antibody has a combination isotype, and if it does have a combination isotype what the combination is. Although no statement appears on the pages on which the example appears, the antibody used in the examples is identified on page 8 of the specification, lines 26-28, as mouse IgG2a/rat IgG2b. Note that the sentence in which this antibody is mentioned begins with the words "As demonstrated in in vitro experiments, ..." The "in vitro experiments" referred to are indeed the in vitro experiments that are reported in the working example.

While the working example uses only one bispecific antibody from among those presently claimed, other portions of the specification describe in general terms the immunization method that is used to obtain the bispecific antibodies within the scope of the invention. Still other portions provide the proper level of guidance concerning how to use the bispecific antibodies. Descriptions of the immunization method are found on page 3, 4th paragraph, to page 13, last paragraph. Specific information on how to use the antibodies is found on page 6, last paragraph, to page 8, second paragraph, which includes detailed descriptions of both the short-term and long-term incubation methods. More specifically, the second paragraph on page 6 describes the use of the antibodies in treating patients by reinfusing the antibodies in patients after the primary tumor has been removed; the paragraph bridging pages 4 and 5 describes the properties of the antibodies; and the section extending from the third paragraph on page 8 to the last paragraph on page 10 explains why the invention is so effective. Further guidance on practicing the invention is found in the section extending from page 11, last paragraph, to page 13, first full paragraph; the preparation of the antibodies is described in detail in the section extending from page 18, next-to-last paragraph, to page 20, last paragraph; and details of the immunization protocols are found in the section extending from page 25, last paragraph, to page 27, next-to-last paragraph. Reference is also made in these sections to prior art documents as evidence that immunization procedures are well known to those skilled in the art. The specification thus provides information sufficiently detailed and extensive that the person skilled in the art is easily able to practice the invention, i.e., to prepare and use the antibodies, over the full scope claimed. As of the effective filing date of

this application, it is simply a matter of routine laboratory procedure to construct and produce antibodies of particular isotypes, including those claimed in the present invention.

The office Action cites the 1995 Lindhofer et al. paper as evidence that usable bispecific antibodies are difficult to prepare and the success of their preparation is unpredictable. Applicants submit that this is not a fair reading of the teachings of the paper. The paper recognizes that the preparation of bispecific antibodies was well known in the art and the purpose of the paper was to describe an improved method that resulted in higher yield and better quality. This does not mean that one could not prepare the antibodies from the previous methods. To use an example in another technology, the PCR method, for example, has been used successfully since its inception and is readily reproduced, but has been improved since and continues to be improved. The same is true for the production and purification of bispecific antibodies: the 1995 Lindhofer et al. paper merely reports an improvement on preexisting technology. Different methods of producing bispecific antibodies for diagnosis and therapy are described in references (1) to (5) of the paper.

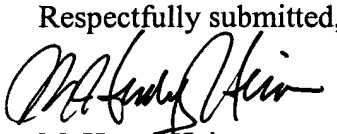
The view expressed in the Office Action that only the rat/mouse isotype combination appears to be known, citing the 1995 Lindhofer et al. paper, is respectfully traversed. While the rat/mouse isotype is indeed the focus of the paper, the paper also acknowledges the existence of conventional mouse/mouse and rat/rat quadromas (see the first paragraph of the paper and the paragraph bridging pages 223 and 224). The point made by the paper is that rat/mouse quadromas have a higher yield (3.5 times) of bispecific antibodies on the average than the conventional mouse/mouse and rat/rat quadromas (see page 224, left column, last paragraph, second sentence). The paper does not state that the rat/mouse quadromas were the only ones known. Increasing the yield of bispecific antibodies is not the aim of the present invention. The present invention focuses on a subclass of bispecific antibodies, and the methods disclosed by the Lindhofer et al. paper as well as those of the references that the paper cites (including those for isotypes other than rat/mouse) are useful in the product of all antibodies of the subclass.

CONCLUSION

For the reasons presented above, Applicants submit that the claims of the present application recite patentable subject matter and that the claims and specification meet all requirements of 35 U.S.C. 103. Accordingly, reconsideration of the application is respectfully requested.

Should any matters remain that can be resolved by a telephone conference, the examiner is requested to telephone the undersigned at 415-576-0200.

Respectfully submitted,



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VERSION WITH MARKINGS TO SHOW CHANGES MADE

1. (three times amended) Method for the preparation of **an antibody-tumor cell preparation** [vaccine] for immunization of humans and animals against tumor cells comprising the steps of:

a) isolating autologous tumor cells;

b) treating the tumor cells to prevent the survival thereof following reinfusion;

c) incubating the thus treated tumor cells with intact heterologous bispecific antibodies showing the following properties:

(i) [▨] binding to a T cell;

(ii) [▨] binding to at least one antigen on a tumor cell;

(iii) [-] binding, by their Fc portion to Fc receptor-positive cells**; and**

(iv) capable of activating the Fc receptor-positive cell whereby the expression of cytokines, co-stimulatory antigens or both is induced or increased,

wherein the bispecific antibodies **have isotype combinations** [are members] selected from the group consisting of [the following isotype combinations]:

rat-IgG2b/human-IgG1,

rat-IgG2b/human-IgG2,

rat-IgG2b/human-IgG3[oriental allotype G3m(st) = binding to protein A],

rat-IgG2b/human-IgG4,

rat-IgG2b/rat-IgG2c,

mouse-IgG2a/human-IgG3[caucasian allotypes G3m(b+g) = no binding to protein A, in the following indicated as *],

mouse-IgG2a/mouse-[VH-CH1,VL-CL]-human-IgG1-[hinge]-human-IgG3*-[CH2-CH3],

mouse-IgG2a/rat-[VH-CH1,VL-CL]-human-IgG1-[hinge]-human- IgG3*-[CH2-CH3],

mouse-IgG2a/human-[VH-CH1,VL-CL]-human-IgG1-[hinge]-human-IgG3*-[CH2-CH3],

mouse-[VH-CH1,VL-CL]-human-IgG1/rat-[VH-CH1,VL-CL]-human-IgG1-[hinge]-human-IgG3*-[CH2-CH3],

mouse-[VH-CH1,VL-CL]-human-IgG4/rat-[VH-CH1,VL-CL]-human-IgG4-[hinge]-human-IgG4[N-terminal region of CH2]-human-IgG3*[C-terminal region of CH2: > aa position 251]-human-IgG3*[CH3],

rat-IgG2b/mouse-[VH-CH1,VL-CL]-human-IgG1-[hinge-CH2-CH3],

rat-IgG2b/mouse-[VH-CH1,VL-CL]-human-IgG2-[hinge-CH2-CH3],

rat-IgG2b/mouse-[VH-CH1,VL-CL]-human-IgG3-[hinge-CH2-CH3, oriental allotype],

rat-IgG2b/mouse-[VH-CH1,VL-CL]-human-IgG4-[hinge-CH2-CH3],

human-IgG1/human-[VH-CH1,VL-CL]-human-IgG1-[hinge]-human-IgG3*-[CH2-CH3],

human-IgG1/rat-[VH-CH1,VL-CL]-human-IgG1-[hinge]-human-IgG4[N-terminal region of CH2]-human-IgG3*[C-terminal region of CH2 : > aa position 251]-human-IgG3*[CH3],

human-IgG1/mouse-[VH-CH1,VL-CL]-human-IgG1-[hinge]-human-IgG4[N-terminal region of CH2]-human-IgG3*[C-terminal region of CH2 : > aa position 251]-human-IgG3*[CH3],

human-IgG1/rat-[VH-CH1,VL-CL]-human-IgG1-[hinge]-human-IgG2[N-terminal region of CH2]-human-IgG3*[C-terminal region of CH2 : > aa position 251]-human-IgG3*[CH3],

human-IgG1/mouse-[VH-CH1,VL-CL]-human-IgG1-[hinge]-human-IgG2[N-terminal region of CH2]-human-IgG3*[C-terminal region of CH2 : > aa position 251]-human-IgG3*[CH3],

human-IgG1/rat-[VH-CH1,VL-CL]-human-IgG1-[hinge]-human-IgG3*-[CH2-CH3],

human-IgG1/mouse-[VH-CH1,VL-CL]-human-IgG1-[hinge]-human-IgG3*-[CH2-CH3],

human-IgG2/human-[VH-CH1,VL-CL]-human-IgG2-[hinge]-human-IgG3*-[CH2-CH3],

human-IgG4/human-[VH-CH1,VL-CL]-human-IgG4-[hinge]-human-IgG3*-[CH2-CH3],

human-IgG4/human-[VH-CH1,VL-CL]-human-IgG4-[hinge]-human-IgG4[N-terminal region of CH2]-human-IgG3*[C-terminal region of CH2 : > aa position 251]-human-IgG3*[CH3],

mouse-IgG2b/rat-[VH-CH1,VL-CL]-human-IgG1-[hinge]-human-IgG3*-[CH2-CH3],

mouse-IgG2b/human-[VH-CH1,VL-CL]-human-IgG1-[hinge]-human-IgG3*-[CH2-CH3],

mouse-IgG2b/mouse-[VH-CH1,VL-CL]-human-IgG1-[hinge]-human-IgG3*-[CH2-CH3],

mouse-[VH-CH1,VL-CL]-human-IgG4/rat-[VH-CH1,VL-CL]-human-IgG4-[hinge]-human-IgG4-[CH2]-human-IgG3*-[CH3],

human-IgG1/rat-[VH-CH1,VL-CL]-human-IgG1-[hinge]-human-IgG4-[CH2]-human-IgG3*-[CH3],

human-IgG1/mouse-[VH-CH1,VL-CL]-human-IgG1-[hinge]-human-IgG4-[CH2]-human-IgG3*-[CH3],

human-IgG4/human-[VH-CH1,VL-CL]-human-IgG4-[hinge]-human-IgG4-[CH2]-human-IgG3*-[CH3], and

rat/mouse.

13. (twice amended) **A method for preparing a vaccine comprising an antibody-tum r cell preparation, said method** [Method according to claim 1, further] comprising preparing **an** [the] antibody-tumor cell preparation **by the method of claim 1, and preparing a** [containing] vaccine **from said antibody-tumor cell preparation**.

14. (twice amended) **A method for preparing a vaccine comprising activated peripheral blood mononucleated cells, said method comprising preparing an antibody-tumor cell preparation by the method of** [Method according to] claim 1[,] in which [in] step (c) **comprises incubating the thus-treated tumor cells with both said intact heterologous bispecific antibodies and peripheral blood mononucleated cells, thereby activating said** peripheral blood mononucleated cells [are added and thereby activated said method further comprising], and (d) preparing a vaccine from the thus-activated peripheral blood mononucleated cells.

17. (twice amended) Method according to claim **1** [14] in which said incubation of step (c) is performed for a period of 1 to 14 days.

18. (twice amended) Method according to claim **1** [14] in which said incubation of step (c) is performed with about 10^8 to 10^{10} mononucleated peripheral cells.

23. (twice amended) Method for **preventing the reoccurrence of a tumor caused by the tumor cells against which the intact heterologous bispecific antibodies of claim 1 are directed, said method** [the prevention and/or treatment of a tumorous disease,] comprising administering to an individual **in whom such tumor cells have appeared** [susceptible to such disease] a tumor cell preparation prepared according to the method of claim 1.

27. (amended) Method according to claim 1 in which said rat/mouse bispecific antibody **has an isotype combination** [is] selected from the group consisting of [the following isotype combinations]:

rat-IgG2b/mouse-IgG2a,

rat-IgG2b/mouse-IgG2b, and

rat-IgG2b/mouse-IgG3.